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New metabolites from the microbial oxidation of fluorinated aromatic compounds [1]

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Abstract

m-Bromo-α,α,α-trifluorotoluene (**1**) and 1-bromo-2,3-difluorobenzene (**4**) were subjected to microbial oxidation by *Pseudomonas putida* strain 39/D and the corresponding *Escherichia coli* recombinant microorganism (strain JM109(pDTG601)), which express toluene dioxygenase. The absolute stereochemistry of the major oxidation products have been determined as *cis*-(2*R*,3*S*)-5-bromo-2,3-dihydroxy-α,α,α-trifluoromethylcyclohexa-4,6-diene (**2**), and *cis*-(2*S*,3*S*)-1-bromo-5,6-difluoro-2,3-dihydroxy-4,6-diene (**5**). The regiochemistry of a minor metabolite has been established as *cis*-5-bromo-3,4-dihydroxy-α,α,α-trifluoromethylcyclohexa-1,5-diene (**3**).

Keywords: Toluene dioxygenase; Biooxidation; Microbial aromatic dihydroxylation; Fluorinated metabolites

1. Introduction

The ability of several bacterial strains to metabolize aromatic compounds via *cis*-diols is well known [1–3]. Some of the most common metabolites, enantiomerically pure cyclohexadiene *cis*-diols, such as those derived from halobenzenes, have proven to be useful in the total synthesis of numerous natural products. Synthetic targets that have been achieved in efficient sequences include conduritols [4], conduramines [5,6], inositols [7,8], prostaglandin precursors [9], alkaloids [10–12], sugars [13,14] and terpenes [15,16].[3]

Well over 200 arene *cis*-diols are known, and new compounds are being isolated by the application of different bacterial strains [26,27]. Although the exact characteristics of the enzyme toluene dioxygenase are not known, it oxidizes substituted aromatics with remarkable specificity while tolerating a wide range of stereoelectronic parameters. For example, a *cis*-diol can be produced from chlorobenzene as well as from styrene, ethylbenzene, and other substituted arenes [27].

The regiochemistry of the oxidation of small monocyclic arenes by means of *Pseudomonas putida* 39/D is predictable in most cases: the diol is generally introduced in the 2,3 position relative to the most bulky substituent. Extensive studies of dioxygenase-catalyzed oxidations have been conducted during the last 25 years [1–3] with substrates ranging from monocyclic aromatic compounds to polycyclic systems such as naphthalene, phenanthrene, benzo[a]anthracene, indole, and other heterocycles [28–36].

The biooxidation of substituted bromobenzenes, chlorobenzenes, and fluoroaromatic compounds is also known to furnish 2,3-diols [37–40], and several studies are available on the oxidation of other disubstituted arenes [41–43]. Whereas trisubstituted aromatics have not been as extensively studied, several examples of biooxidation of these compounds can be found in the literature [44–46]. In an effort to determine the likely topology of the active site and to learn about the limits and specificity of the enzyme, we initiated the study of *m*-disubstituted and trisubstituted fluorine-containing aromatics. Such fluorinated compounds can be useful for studying the complete metabolic pathway of the degra-

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[1] Taken in part from the MS thesis of Michele Stabile, Virginia Polytechnic Institute and State University, 1993.

[2] Preliminary experiments performed at Virginia Tech by these authors.

[3] For comprehensive reviews of cyclohexadiene-*cis*-diol chemistry see Refs. [17–25].

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Table 1
Fluorine-containing metabolites obtained from toluene or toluate dioxygenase transformations [49–62]

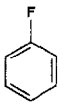
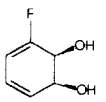
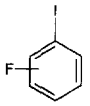
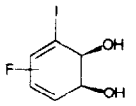
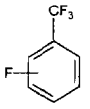
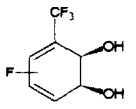
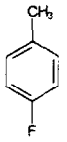
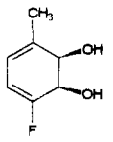
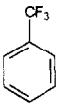
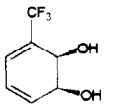
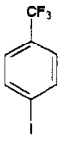
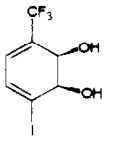
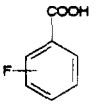
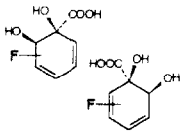
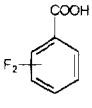
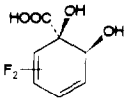
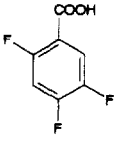
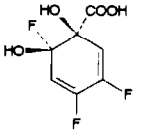
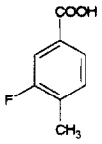
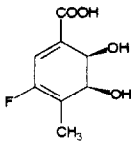
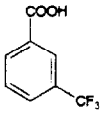
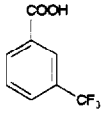
Entry	Substrate	Product(s)	Microorganism	Ref.
1			*P. putida* UV4	[49]
2–4	 *o*-fluoro *m*-fluoro *p*-fluoro		*P. putida* UV4 *E. coli* JM109(pDTG601)	[50]
5–6	 *o*-fluoro, *p*-fluoro		*P. putida* NCIB 12190	[51]
7			*P. putida* 39/D *P. putida* UV4	[51–53]
8			*P. putida* UV4	[49,51]
9			*P. putida* UV4	[52]
10,11	 *o*-fluoro, *m*-fluoro		*A. eutrophus* B9 *Pseudomonas sp* B39	[54–58]
12,13	 3,4-difluoro, 3,5-difluoro		*P. putida* JT103	[59,60]
14*			*P. putida* JT103	[60]

Table 1 (*continued*)

Entry	Substrate	Product(s)	Microorganism	Ref.
15			*P. putida* PL-pT-11/43	[61]
16			*P. putida* mt-1 *A. eutrophus* B9	[62]

*The structure of the metabolite was suggested from the degradation products obtained from defluorination.

dation of aromatic compounds by toluene dioxygenase and in the preparation of fluorinated carbohydrates for medical applications [47]. In a recent example [48], the biooxidation of fluorobenzoic acids has been studied by direct analysis of the crude cell-free centrifugate by ^{19}F NMR. Optically pure fluorine-containing diene diols of the type listed in Table 1 may find use in the preparation of fluorodeoxysugars and inositols, compounds that are useful in evaluating metabolic pathways [63,64]. Although a number of fluorinated aromatic compounds have been subjected to microbial biooxidation, the list is small compared to the number of metabolites known for other arenes (see Table 1). In this manuscript we report the isolation and identification of three new metabolites derived from biooxidation of *m*-bromo-α,α,α-trifluorotoluene (**1**) and 1-bromo-2,3-difluorobenzene (**4**).

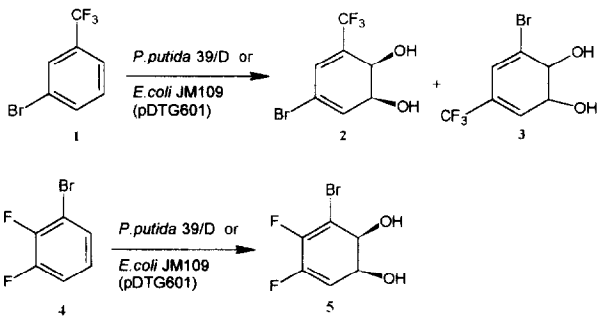
2. Results and discussion

2.1. Isolation, purification, and regiochemistry of the new compounds

In the experiments with *P. putida* 39/D, the microorganisms were grown aerobically in a 10-l fermentor and induced with toluene under conditions previously reported [65]. The substrates were supplied to the cells grown in liquid medium. Centrifugation and extraction of the broth provided new compounds from the biotransformation of compounds **1** and **4** (Scheme 1).[4]

2.1.1. Biooxidation of m-bromo-α,α,α-trifluorotoluene

In the biotransformation of **1**, compounds **2** and **3** were obtained in a ratio of 25:1 in a combined yield of 50 mg/l. Purification of **2** and **3** was accomplished by reversed-phase HPLC. After flash chromatography of the crude product mixture from the biotransformation of substrate **1** on silica gel (10% deactivated with water), the pure products were identified as 2,3-toluene diol (from the induction period),[5] **2**, and **3**. The melting points of the compounds were 112–113°C and 145–146°C, respectively, after recrystallization (dichloromethane:hexanes).



Scheme 1.

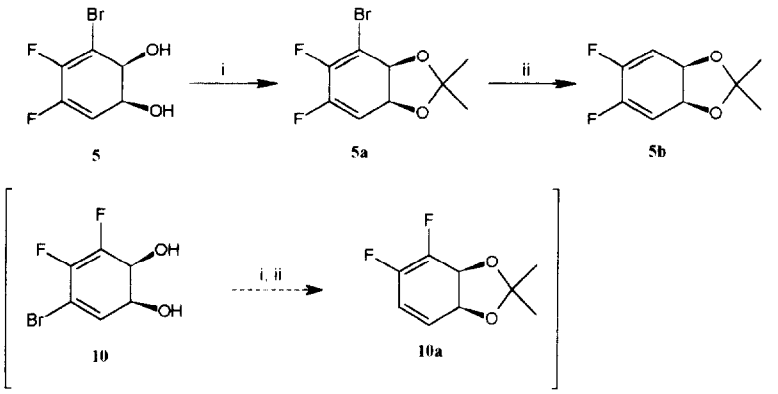
The microbial oxidation was repeated with the recombinant *Escherichia coli* strain JM109(pDTG601). Cells were grown aerobically in a 12-l fermentor, followed by induction with isopropyl-β-d-thiogalactopyranoside (IPTG). In this case the ratio of **2** and **3** was 97:3. Only **2** can be recovered pure by silica gel chromatography (1:1 hexanes/EtOAc as eluent) but **3** can be concentrated by collecting the early fractions from the column. Reversed-phase HPLC (Microsorb C18 column, MeOH:H_2O/60:40, retention times: t_r(**2**) = 11.22 min; t_r(**3**) = 9.62 min) of this fraction afforded pure **3**.

2.1.2. Biooxidation of 1-bromo-2,3-difluorobenzene

In the oxidation of compound **4**, only toluene diol and compound **5** were recovered. The isolated yield of the latter was 50 mg/l, and the melting point after recrystallization (ethyl ether) was 104.5–105.5°C. Substrate **4** was also oxidized on large scale by means of *E. coli* JM109(pDTG601). In this case metabolite **5** was obtained with a yield of 0.7 g/l as the only product.

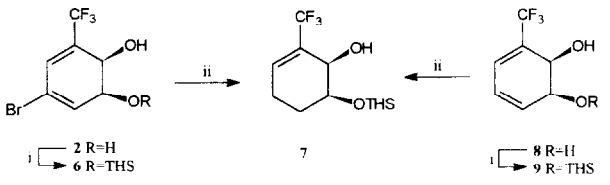
[4] After the fermentation is complete the broth is centrifuged to remove the cells and the pH adjusted to 8.5. The broth is then saturated with NaCl and extracted several times with ethyl acetate to obtain the crude diol mixture.

[5] *P. putida* strain 39/D requires induction with a suitable compound before the oxidizing enzyme is produced. In this study, toluene vapor (a known inducer) was bubbled through the broth before the corresponding aromatic substrate was introduced.



i. DMP, TsOH (cat.) Acetone, r.t., 1 h. ii. a) *t*-BuLi, THF, -78°C. b) MeOH

Scheme 2.



i = imidazole, THSCl, CH_2Cl_2 ii = H_2, PtO_2, MeOH

Scheme 3.

The regiochemistry in the oxidation of compound **4** was confirmed by chemical means. In a voluntary action of 'chemical vandalism' (i.e., the wilful destruction of chirality), the derivative **5a** was transformed into the *meso* compound **5b** by reductive dehalogenation (Scheme 2). The alternative structure (**10**) was ruled out, because, if subjected to the same dehalogenation procedure, it would have yielded the optically active compound **10a**, therefore, the structure of the bromodifluorodiol obtained from the biotransformation was assigned as **5**.

2.2. Determination of the absolute stereochemistry

The absolute stereochemistry of the new diols obtained was studied by two methods. First, metabolite **2** was converted to compound **7** (Scheme 3), which was independently prepared from the known standard **8** (entry 8, Table 1). The diol was first protected with dimethylthexylsilyl chloride (THSCl) and imidazole in CH_2Cl_2 and hydrogenated with Adams' catalyst in methanol and triethylamine to afford **7**.

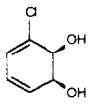
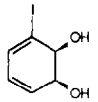
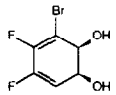
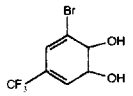
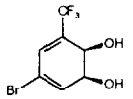
i=imidazole, THSCl, CH_2Cl_2 ii=H_2, PtO_2, MeOH

Direct comparison of the optical properties of compound **7** prepared from either **2** or **8** confirmed the absolute stereochemistry of the major metabolite as shown. Since the values obtained for the optical rotation were identical within experimental error, and since the % ee of diol **8** is known to be >98%, the % ee for diol **2** was assumed to be >98% as well.

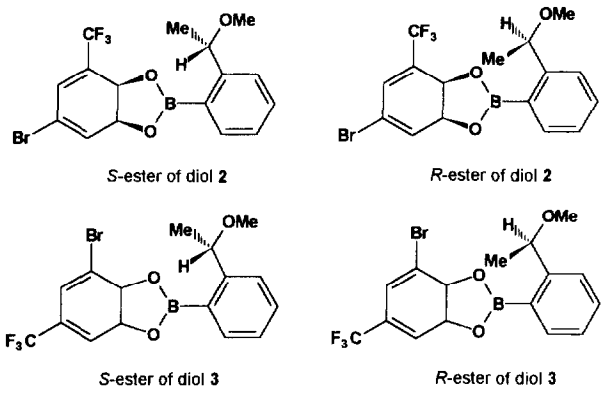
To determine the absolute stereochemistry of **3** (entry **d**, Table 2) from the very small amounts available from microbial oxidations, derivatization with chiral boronic acid was used. This method was successfully used to determine the absolute stereochemistry and % ee of diol metabolites present in small amounts (e.g., 2 mg) [66]. For a series of monosubstituted arene *cis*-diols of known % ee and absolute stereochemistry, it was observed that ^{1}H NMR analysis of the chiral boronate esters provided well-resolved diastereomeric methyl and methoxy signals if a suitable solvent was used. With the *R*- and *S*- boronic acid in hand, the ^{1}H NMR signals of either diastereomeric ester can be correlated to the corresponding parental acid. We report here the first attempt to apply this method to di- and tri- subtituted arene *cis*-diols.

Resnick et al. [66] studied a series of diastereomeric boronate esters prepared from *R*- and *S*- boronic acids (Scheme 4). Apparent from Table 2 for entries **a–c**, is a uniform directional shift for methyl and methoxy signals for each diastereomer. The esters made from *S*-boronic acid have the chemical shifts given in Table 2. The corresponding chemical shift for the methoxy signal of the *R*-esters is consistently upfield for X = Cl. I relative to this value thus giving a positive $\Delta\delta$ ppb. For example, taking **a**, the δ_{OMe} for the *S*-ester is 3.175, then for the *R*-ester is 3.159, giving $\Delta\delta$ ppb equal to +16 ppb. Comparing the methyl signals, that for the *R*-ester is consistently downfield relative to the signal for the *S*-ester. The same trend is followed by **b** and **c**, which indicates unambiguously that compound **5** (entry **c**) has the same absolute configuration as entries **a** and **b** above. Compound **3** (entry **d**) reverses this trend and the assignment of the alternative absolute configuration of **3b** could therefore be given to this molecule (Scheme 5). However, if the assignment was made as **3b**, it would be the first instance of 'reversed' enantioselectivity in dioxygenase-mediated dihydroxylations known to date. Because of the small amount (3% content in fermentations), it was not possible to perform a full degradation to a known standard, such as the oxidative cleavage to the known protected erythruronolactone **11** [67,68], which would provide an unambiguous answer (Scheme 6). Until direct methods of assignment are performed, we would prefer not to commit to absolute stereo-

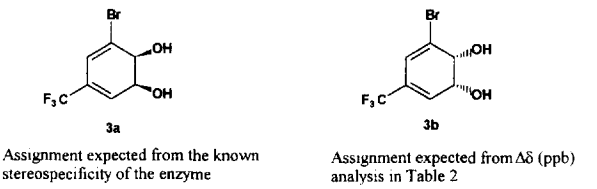
Table 2
Directional chemical shifts obtained for the boronate ester derivatives

Entry[a]	Diol	Data for the (−)-S derivative of the corresponding diol[b]				
		% ee	–OMe		$-CH_3$	
			δ (ppm)	Δδ (ppb)	δ (ppm)	Δδ (ppb)
a		>98	3.175	+16	1.551	−29
b		>98	3.207	+23	1.573	−6
c		>98	3.144	+23	1.519	−25
d		>98	3.137	−29	1.569	+23
e		>98	[3.225]	−9	[1.418]	+9
f		>98	[3.218]	−17	[1.412]	+19
g		>98	[3.232]	−19	[1.429]	+25

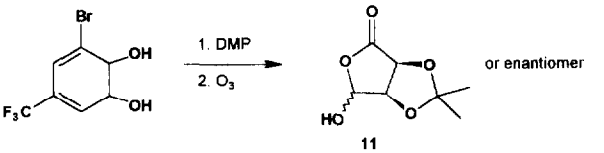
[a]Entries a,b,e, and f were reported from Ref. [66].
[b]Boronate esters were analyzed in C_6D_6 or [$CDCl_3$].



Scheme 4.



Scheme 5.



Scheme 6.

chemistry assignment for **3** based only on the ^1H NMR analysis of boronic esters, especially those derived from *m*-disubstituted arene *cis*-diols for which sufficient correlations are not available.

Compound **2** (entry **g**) was analyzed similarly using entries **e** and **f** as references. The results for the boronate ester from this diol follow the trend set by the references with respect to positions of chemical shifts for diagnostic methyl and methoxy signals. Thus compound **2** can be assigned the same absolute stereochemistry as **e** and **f**, as confirmed by the comparison with reduced diol **7**.

Other standard diols (e.g., fluorobenzene, etc.) were studied by the chiral boronate ester methodology and all followed the same trend. Although used only for monosubstituted

arene *cis*-diols, extrapolation to disubstituted diols might be justified, provided other direct methods are also used. The result for compound **3** is questionable on the basis of known mode of stereofacial oxidation of arenes by toluene dioxygenase from the literature. The enantiomeric excess given in each entry was based on the assumption that the other enantiomer could have given distinct diastereomeric signals if it was present and that the signals would be resolved.

In conclusion, the fluorinated disubstituted arenes **1** and **4** were metabolized by toluene dioxygenase to provide the *cis*-diols **2** and **5** in high yield and enantiopurity. Applications of these compounds in synthesis will be reported in the future.

3. Experimental details

All nonhydrolytic reactions were carried out under an argon atmosphere with standard techniques for the exclusion of air and moisture. Glassware used for moisture-sensitive reactions was flame-dried under vacuum. Analytical TLC was performed on Silica Gel 60F-254 plates. Flash chromatography was performed on Kieselgel 60 (EM Reagents, 230–400 mesh). Mass spectra were recorded on a Varian MAT-112 instrument (low resolution) or on a double-focusing VG 7070 E-HF instrument (exact molecular ion mass). Infrared spectra were recorded on a Perkin-Elmer 283B or 710B instruments. HPLC was performed on Microsorb 5 μm C18, 4.6 mm ID × 25 cm l (analytical) and Microsorb 5 μm C18, 21.4 mm ID × 25 cm l (preparative) columns. Proton NMR spectra were obtained on Bruker WP-270 or Varian UN-400 instruments. Attached proton test (APT) experiments were conducted to determine C multiplicities. Optical rotations were recorded on a Perkin-Elmer 241 digital polarimeter.

3.1. Typical procedure for microbial transformations

E. coli JM109(pDTG601) was grown overnight at 35°C in an enriched medium containing ampicillin (100 mg/l) [34]. The preculture was then transferred to a 12-l fermentor containing 8 l of a similar medium, and the cells were grown to an OD = 70 (λ = 660 nm). The liquid substrates were added dropwise to the culture, and the metabolic transformation of the substrates was monitored by observing the oxygen consumption and CO_2 production by the culture. Diol production was checked by measuring the characteristic absorbance peak in the UV region (λ = 270 nm). After all metabolic activity ceased (or no more diol formation was observed), the fermentation was stopped and the pH was adjusted to 8.4 with NaOH. The cells were separated from the broth by centrifugation, and the resulting clear solution was saturated with NaCl and extracted with base-washed (saturated $NaHCO_3$) EtOAc. The organic layer was dried with Na_2SO_4, and the solvent was evaporated. The crude diol was purified via flash chromatography on deactivated silica gel (10% water) using hexanes/EtOAc as eluent (7:3) to give either a mixture of **2** (0.7 g/l) and **3** (20 mg/l) or pure compound **5** (0.7 g/l).

3.1.1. Microbial oxidation of m-bromo-α,α,α-trifluorotoluene (1)

cis-(2*R*,3*S*)-5-Bromo-2,3-dihydroxy-α,α,α-trifluoromethylcyclohexa-4,6-diene (**2**). R_f = 0.4 (hexanes/EtOAc 1:1) mp 112–113°C (CH_2Cl_2/hexanes) $[\alpha]_D^{25} = -18.1$ (*c* 0.53, MeOH) IR ($CHCl_3$) 3441, 1636, 1270, 1173, 1052, 1025 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.65 (m, 1H), 6.37 (s, 1H), 4.46 (s, 1H), 4.32 (d, *J* = 5.9, 1H), 2.53 (br s, 2H). ^{13}C NMR (CD_3OD) δ 138.1 (C), 132.2 (CH), 131.4 (C), 124.5 (C), 113.6 (CH), 72.3 (CH), 63.9 (CH) MS (CI+) *m/z* (rel. int.) 259(19), 214(100), 159(33), 133(70), 83(42). Calcd. for $C_7H_6BrF_3O_2$: C, 32.46; H, 2.34; found: C, 32.43; H, 2.34.

cis-(3*S*,4*S*)-5-Bromo-3,4-dihydroxy-α,α,α-trifluoromethylcyclohexa-1,5-diene (**3**). R_f = 0.4 (hexanes/EtOAc 1:1) mp 145–146°C $[\alpha]_D^{25} = -46.5$ (*c* 0.66, MeOH) IR ($CHCl_3$) 3300, 3200, 2920, 1580, 1550, 1260, 750 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.48 (d, *J* = 6, 1H), 6.42 (dd, J_1 = 6.2, J_2 = 6.0, 1H), 4.51 (m, 2H), 2.78 (s, 1H), 2.29 (s, 1H) ^{13}C NMR (400 MHz, CD_3OD) δ 136.3 (CH), 129.4 (C), 128.4 (C), 125.5 (C), 125.1 (CH), 73.4 (CH), 66.9 (CH) MS (EI) *m/z* (rel. int.) 258(20), 240(14). HRMS calcd. for $C_7H_6F_3O_2$: 257.9503; found: 257.9505, Δ = 0.1 mmu.

3.1.2. Microbial oxidation of 1-bromo-2,3-difluorobenzene (4)

cis-(2*S*,3*S*)-1-Bromo-5,6-difluoro-2,3-dihydroxy-4,6-diene (**5**). R_f = 0.4 (hexanes/EtOAc 1:1) mp 104.5–105.5°C (ethyl ether) $[\alpha]_D^{25} = 34.6$ (*c* 0.49, MeOH) IR (KBr) (cm^{-1}): 3910(w), 1630(m), 1380(m), 1220(s), 1090(s), 1015(s). ^1H NMR ($CDCl_3$) δ 5.12 (m, 1H), 4.60 (br s, 1H), 4.47 (br s, 1H), 2.66 (br s, 1H), 2.40 (br s, 1H). ^{13}C NMR (C_3D_6O) δ 148.46 (dd, J_1 = 259.0 Hz, J_2 = 26.3 Hz, C), 148.36 (dd, J_1 = 258.6 Hz, J_2 = 28.2 Hz, C), 109.21 (dd, J_1 = 9.9 Hz, J_2 = 1.5 Hz, CH), 106.77 (dd, J_1 = 13.3 Hz, J_2 = 3.4 Hz, C), 72.99 (d, *J* = 2.3 Hz, CH), 67.68 (d, *J* = 8.4 Hz, CH). ^{19}F NMR ($CDCl_3$): δ (ppm) −124.25 (broad s), −132.84 (broad s). MS *m/e* (rel. int.) 228(24, M^+ ($C_6H_5O_2{}^{81}BrF_2$)), 226(19, M^+ ($C_6H_5O_2BrF_2$)); 130(16); 127(21); 101(100). HRMS calcd. for $C_6H_5O_2BrF_2$: 225.9441; found: 225.9477, Δ = 3.6 mmu. Calcd. for $C_6H_5BrF_2O_2$: C, 31.75; H, 2.22; found: C, 31.46; H, 2.19.

3.1.3. Preparation of derivatives of the isolated metabolites

cis-(2*R*,3*S*)-5-Bromo-3-dimethylsilylthexyl-2-hydroxy-α,α,α-trifluoromethyl-4,6-cyclohexadiene (**6**). To a stirred solution of **2** (80 mg, 0.31 mmol) in CH_2Cl_2 (10 ml) was added imidazole (26 mg, 0.39 mmol) and dimethylthexylsilyl chloride (0.073 ml, 0.37 mmol). The mixture was stirred at 0°C overnight. The solution was equilibrated to room temperature, dried over Na_2SO_4 and evaporated to yield a colorless oil **6** (114 mg, 92% yield). R_f = 0.6 (hexanes/EtOAc

10:1) $[\alpha]_D^{25} = 9.2$ (*c* 0.97, MeOH) IR (neat) 3448, 2960, 1168, 1130, 1050, 1028 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.54 (m, 1H), 6.24 (m, 1H), 4.43 (m, 1H), 4.20 (m, 1H), 2.66 (d, $J = 4.2$, 1H), 1.64 (m, 1H), 0.91 (m, 6H), 0.18 (d, $J = 5.3$, 3H) ^{13}C NMR (400 MHz, $CDCl_3$) δ 134.5 (C), 131.0 (CH), 129.5 (C), 122.7 (C), 114.3 (CH), 71.0 (CH), 63.7 (CH), 34.1 (C), 25.2 (C), 20.2 (CH_3), 20.0 (CH_3), 18.5 (CH_3), 18.4 (CH_3), −2.54(CH_3), −3.08(CH_3). MS *m/z* (rel. int.) 383(90). HRMS calcd. for $C_{15}H_{24}BrF_3O_2Si$ (M–OH): 383.0653; found: 383.0684, $\Delta = 3.1$ mmu.

cis-(2*R*,3*S*)-3-Dimethylsilyl-2-hydroxy-α,α,α-trifluoromethyl-6-cyclohexene (**7**). An autoclave was charged with **9** (200 mg, 0.62 mmol) dissolved in MeOH(10 ml). Two drops of triethylamine and catalytic amount of PtO_2 were added to this solution. The apparatus was pressurized to 85 psi with H_2 and allowed to stir for 2.5 h. After filtration through Celite and evaporation of the solvent, a crude oil was obtained. Purification via silica gel chromatography afforded a colorless oil (51 mg, 26% yield). The same compound was obtained by hydrogenation of **6** in 27% yield. $R_f = 0.5$ (hexanes/EtOAc 10:1) $[\alpha]_D^{25} = -60$ (*c* 0.77, MeOH) IR (neat) 3550, 2960, 1660, 1560, 1370 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.52 (s, 1H), 4.19 (s, 1H), 3.81 (dt, $J = 11.2$, $J = 7.2$, 1H), 2.73 (d, $J = 2.4$, 1H), 2.34 (m, 1H), 2.20 (m, 1H), 1.90 (m, 1H), 1.64 (m, 2H), 0.91 (d, $J = 2.4$, 3H), 0.89 (d, $J = 2.4$, 3H), 0.87 (m, 6H), 0.16 (s, 6H) ^{13}C NMR (400 MHz, $CDCl_3$) δ 135.1 (CH), 129.0 (C), 124.1 (C), 69.9 (CH_3), 64.1 (CH), 34.2 (C), 29.6 (C), 24.4 (CH_2), 23.9 (CH_2), 20.2 (CH_3), 20.1 (CH_3), 18.6 (CH_3), 18.5 (CH_3), −2.4 (CH_3), −2.9 (CH_3) MS (FAB) *m/z* (rel. int.) 325(100), 239(90). HRMS calcd. for $C_{15}H_{27}F_3O_{23}Si$: 325.1801, found: 325.1780, $\Delta = 0.1$ mmu.

cis-(2*S*,3*S*)-1-Bromo-5,6-difluoro-2,3-[(isopropylidene)dioxy]cyclohexa-4,6-diene (**5a**). To a stirred solution of **5** (1.13 g, 5.0 mmol) in acetone (100 ml) at room temperature was added neat dimethoxypropane (1.5 ml, 16.8 mmol) dropwise. The solution was stirred for 3 min and TsOH (45 mg, 0.25 mmol) was added. The reaction was stirred for 2 h and another 1.5 ml of DMP was added. After one additional hour the reaction was complete (monitored by TLC). The reaction was diluted with CH_2Cl_2 and then quenched with a saturated solution of Na_2CO_3. The layers were separated and the water layer was extracted three times with CH_2Cl_2. The combined organic extracts were dried with $MgSO_4$, and the solvent removed. The resulting oil was purified by column chromatography (hexanes/EtOAc 1:1) to yield **5a** as a colorless oil. (92%) $[\alpha]_D^{25} = 76.9$ (*c* 1.0, $CHCl_3$); IR (neat): 2949, 1023, 1197, 1364, 1614; ^1H NMR (300 MHz) δ 5.63 (1H, m), 4.85 (2H, m), 1.43 (3H, s), 1.40 (3H, s); ^{13}C NMR δ 149.6 (dd, $J_1 = 263$, $J_2 = 22$, C), 103.7 (d, $J = 16$, C), 102.8 (d, $J = 14$, CH), 75.8 (s, CH), 71.0 (d, $J = 10$, CH), 26.6 (CH), 24.8 (CH) ^{19}F NMR ($CDCl_3$, 300 MHz) δ (ppm) −128.95(m), −124.11(m) MS (EI, 70 eV) *m/z* (rel. int.) 268(38, $M^+ + 2H(C_9H_{11}{}^{81}BrF_2O_2)$), 267(39, $M^+ + 2H(C_9H_{11}{}^{79}BrF_2O_2)$), 253(85), 251(83), 211(97), 209(100), 130(69). HRMS calc. for $C_9H_{11}BrF_2O_2$: 266.9832 ($M^+ + 2H$); found: 267.9885, $\Delta = 2.6$ mmu.

cis-(2*S*,3*S*)-5,6-Difluoro-2,3-[(isopropylidene)dioxy]-cyclohexa-4,6-diene (**5b**). To a stirred solution of **5a** (0.42 g, 2.27 mmol) in anhydrous THF (30 ml) at −78°C was added *tert*-BuLi (1.7 M in pentane, 4.1 ml, 3.4 mmol) dropwise. The solution was stirred for 15 min and MeOH (2.1 ml, 74 mmol) was added dropwise while the temperature was maintained at −78°C. The mixture was stirred for another 15 min and allowed to warm to room temperature. The reaction was diluted with EtOAc, neutralized with NH_4Cl solution, and extracted twice with EtOAc. The organic extract was dried with anhydrous $MgSO_4$, and the solvent was removed at room temperature under reduced pressure. The resulting oil was purified by column chromatography (hexanes/$CHCl_3$ 3:1) to yield **5b** as a yellow oil. (60%) IR (neat) 2989(w), 2936(w), 1736(m), 1652(w), 1404(s), 1244(s), 1212(s), 1176(w), 1047(s), 867(w), 759(s); ^1H NMR (300 MHz) δ (ppm) 5.55 (2H, m), 4.85 (2H, m), 1.45 (3H, s), 1.38 (3H, s); ^{13}C NMR δ (ppm) 150.3 (dd, $J_1 = 262$ Hz, $J_2 = 28$ Hz, C), 106.2 (s, C), 104.6 (dd, $J_1 = 11$ Hz, $J_2 = 3$ Hz, CH), 71.1 (broad s, CH), 27.1 (CH_3), 25.0 (CH_3). ^{19}F NMR (300 MHz, $CDCl_3$) δ (ppm) −131.87 (m, $J = 4.9$ Hz) GC–MS (CI) *m/z* (rel. int.) 188(9, M^+), 130(21), 117(100) 111(76). HRMS $C_9H_{10}F_2O_2$: 188.0649; found: 188.0655, $\Delta = 0.6$ mmu.

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